Transplantation Research in Small Animal Models

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Transplantation research is a discipline that largely benefits from the use of animal models with mouse and pig models being the most frequently used models in organ transplantation research. A suitable animal model should reflect best the situation in humans, and the researcher should be aware of the similarities as well as the limitations of the chosen model. Small animal models with rats and mice are contributing to the majority of animal experiments with the obvious advantages of these models being easy handling, low costs, and high reproductive rates.

small animal model large animal model solid organ transplantation

1. Kidney Transplantation

The rat kidney transplantation model is widely used for acute and chronic rejections as well as to study renal hypertension [1][2]. This experimental procedure has been standardized, and various new techniques contributed to the reduction of complications and therefore decreasing the number of animals needed [1][2][3][4]. Due to this, high long-term survival rates can be achieved with excellent vascular patency rates of up to 95% [5][6]. The orthotopic rat transplantation model is also a reproducible and reliable model to study different aspects of kidney transplantation such as acute, chronic, cellular, or antibody-mediated rejection [3][7].

Mouse models of vascularized kidney transplantation have been widely used to study the mechanism of IRI and transplant rejection. Removing the recipient's native kidneys 4–5 days after transplantation decrease the probability of mortality during the early post-transplant phases due to delayed graft function. Nevertheless, the advantage of removing both native kidneys during the transplantation operation is that the immediate renal graft function can be monitored [8][9][10][11].

In brief, the donor's kidney is harvested for transplantation. For recipient procedures, transplant and bilateral native kidney removal is done either simultaneously [12][13] or some days after transplantation [9][11]. The native kidney is removed first after opening of the abdomen; then, the donor kidney is transplanted.

However, graft loss can still be high due to arterial thrombosis because of turbulences in the blood flow leading to platelet activation and thrombus formation. A revised surgical technique using a cuff for a longer anastomosis and a straighter blood flow resulted in lessened thrombosis and therefore a better outcome. This surgical model provides a fully vascularized orthotopic kidney transplantation model, which can be used for graft tolerance and rejection studies, delayed graft function, and ischemia reperfusion injury studies [12][14].

Syngeneic and allogeneic mouse models have been used for testing IRI treatments in kidney transplantation models. However, the contribution of the individual mouse genetic background should be taken into account when planning and designing studies [8].

Despite the genetic disparities as mentioned above, and even considering that the rodent model is less expensive and better to manage, the direct translatability to human is limited also because of the anatomical differences between these two species. While humans and pigs have a multilobular and multipapillary kidney architecture, mice, rats, dogs and rabbits have unilobular, unipapillary kidneys [15].

2. Liver Transplantation

Animal models contributed significantly to the medical advances of liver transplantation. Although technically even more demanding, as the mouse liver vessels are eight times smaller than those of the average adult rat, a mouse orthotopic liver transplant model has been established. Mouse models are attractive because of their well-characterized genome and the possibility of knockout or transgenic models as well as the lower costs and the continuous availability of research substances [16].

In 1979, Naoshi Kamada pioneered in publicizing the first orthotopic rat liver transplantation using a cuff technique. This technique is well described and standardized and has been used worldwide since then [17][18][19][20][21][22]. During a 5-year period, Kamada performed more than 500 rat liver transplantations with a survival rate of 95.3% [17][18].

The cuff procedure shortens the anhepatic phase in the recipient animal, but it can be associated with foreign body reactions to the cuff [17].

Since then, alternative techniques such as cervical liver transplantation have emerged, but the Kamadas model remained the gold standard. For the rat, a literature review revealed 30 techniques or technical modifications [23].

The mouse model of orthotopic liver transplantation is especially attractive due to its similarity to man. Mice do have unlike rats a gallbladder, and they share a greater similarity between their histocompatibility complexes [16]. This model can give a comprehensive insight in research questions regarding immunobiology and pathobiology of the liver, tissue injury, regulation of alloimmunity, graft rejection, and tolerance induction, as well as liver biology and the pathogenesis of specific liver diseases.

In summary, the orthotopic liver transplantation in the mouse model is a useful tool in transplantation research and can contribute to new therapeutic interventions in this field.

3. Heart Transplantation

Heterotopic vascularized heart transplantation in a rodent model is a frequently used procedure to study the pathogenesis of graft rejection, immune response, the effects of IRI and graft preservation, as well as immunosuppressive regimes [24][25].

During this procedure, the heart of the donor animal is explanted and transplanted heterotopically into the abdomen of the recipient animal with the donor's ascending aorta anastomosed to the recipients infrarenal aorta, and the donor's pulmonary artery anastomosed to the recipient's inferior vena cava [26][27][28][29]. This recipient surgical procedure can also be performed using cervical vessels. In brief, the right cervical common carotid artery and external jugular vein of the recipient are isolated, and a Teflon cuff (Heron cuff technique) is placed over the caudal carotid artery and jugular vein. The donor aorta and pulmonary artery are connected to the cannula of the recipient cervical common carotid artery and external jugular vein and are ligated and fixed. If the transplanted heart is beating, the cervical incision can be closed [30][31].

It is a unique feature of this organ transplant model that graft monitoring can be done directly via palpation of pulsation in the abdomen without the need for repeated blood collection and blood chemistry [24].

This heart transplantation model is not life sustaining, and graft survival is not equated with animal survival. Acute rejection is defined by no palpable graft pulsation, and long-term survival is defined by viability of the graft beyond day 100 after transplantation. With chronic rejection, the heart rate decreases, the pulsation reduces linearly, and the heart size decreases. Animal death at or before day three is considered to be a technical failure [24]. Especially, the analysis of rejection processes in different mouse strains with defined genetic backgrounds offers the unique possibility to gain a deeper insight into immune interactions between receptors and ligands and their effect on physiological and pathophysiological mechanisms of acute and chronic graft rejection [29].

Heterotopic heart transplantation as an in vivo tool has been proven to be a solid vascularized immunological model in acute rejection or chronic transplant failure. As a result of the size, functional orthotopic heart transplantation cannot be performed in mice. In the heterotopic transplant, the ascending aorta is anastomosed to the abdominal aorta and the pulmonary artery is anastomosed to the vena cava. This is leading to a sufficient perfusion of the organ but only to a reduced workload of the graft [32].

Anatomical differences between man and mice regarding the vascular bed are limiting the use of this model for antibody-mediated rejection research. The human coronary arteries are supplied by vasa vasorum, the murine are not, and it is possible that these small vessels provide a route for inflammatory cells and cytokines into the coronary arteries of heart transplants in humans, but not in mice. Additionally, in the human heart, the coronaries are located on the surface, embedded in epicardial fat, and epicardial fat produces proinflammatory cytokines including IL-6, IL-8, and MCP 1. In contrast to humans, in mice, only the first few millimeters of the coronary arteries are on the surface of the heart, and after that short distance, they pass through the myocardium [29][33].

However, keeping these differences in mind, the in vivo model of heterotopic heart transplantation in rodents is a valuable tool with good experimental reproducibility.

4. Lung Transplantation

Although experiments on canine models laid the foundation for the first successful lung transplantation in humans, small animal models contributed significantly to gain further insight into the immune response to pulmonary grafts. However, eventually, the opportunity to successfully perform orthotopic vascularized lung transplantation in mice together with genetic tools paved the way to study in depth the immune and non-immune factors leading to lung graft rejection [34].

The rodent lung transplantation model is complex but meanwhile standardized and well replicable. This model gave insights into lung preservation models, ischemia–reperfusion injury, and the mechanisms of acute and chronic rejection [35].

Lung transplantation is also described in a rat model, but the lack of specific antibodies compared to mouse models restricts the broader use of it. As already described for other organ transplantation models, the possibility of using a large variety of transgenic and knockout strains in mice and many available antibodies outweighs the seriously complicated surgery due to the size [36].

However, still, the rat model did its contributions, as the four phases of acute lung transplant rejection were defined in a rat lung transplantation model, indicating that bronchus-associated lymphoid tissue (BALT) within the lung graft played an important part in accelerating acute rejection [34]. BALT is expressed constitutively in rat lungs but not in mouse or human lungs where it can be induced after inflammatory processes. Due to the morphological differences in fibrotic airway obstruction in humans and rats, the orthotopic lung transplantation model in the rat is not broadly used.

5. Large Animal Models

Small animal models are the model of choice for proof of concept studies and protocol development but unfortunately often do not translate to clinical use. In addition, because rodent models highly depend on the surgeon’s skills, therefore, they are not easy reproducible. Large animal models, especially in transplantation medicine, are an important element for establishing preclinical models, which often translate to the clinic [37].

For a long time, advances in transplantation have been based on the use of large animal models by bridging the gap between inbred mouse experimentation and the translation into human clinical trials [38].

Large animal models include sheep, goats, cows, pigs, horses, dogs, and primates. Among these, the most commonly used species are canines, swine, and nonhuman primates (NHPs) [39][40]. They all have similarities to human physiology and anatomy, which makes them suitable for preclinical studies. The dog model contributed significantly toward kidney transplantation, as the vessel anastomosis were established in this model [15].

However, the usage of pigs is driven by some beneficial facts: the pig is rated as a food source and therefore less ethical, and public resentments accompany the use of this species in research compared to dog or nonhuman primate models. As a result of their size, repeated sample taking is possible. In addition, pigs can be genetically modified, and the pigs genome has a high homology with humans [15]. Another advantage of the pig model is the similarly structured MHC Complex in comparison to humans. This is especially important in transplantation research [41][42]. Large animal models are of great interest in transplantation immunobiological research, as MHC class II expression levels and regulation of the endothelium is similar to humans. Therefore, dogs, pigs, and NHPs are frequently used in transplantation research, as their anatomy and immunobiology is close to that of humans [43].

The advantages of large animals compared to small animal models lays in the greater similarity of physiologically and anatomically characteristics such as size, tissue structure, and life expectancy. An important aspect is also that large animal species usually are outbred populations, which mimics more accurately the diversity of the human population.

Compared to small animal models, large animals provide a more clinically relevant model to test the translatability of new therapies [39][40].

A variety of large animal species have previously been used, but the necessity for highly specific and tailored therapies demands a model with a significant grade of homology to humans. As a consequence, nonhuman primates became the preferred choice in many experimental settings [38].

Although being the model that resembles the situation in humans at its best, the use of NHPs is often limited by increased regulatory requirements and ethical concerns. The high demands on husbandry and training, zoonotic risks, and the need for personal protective equipment are making these experiments extremely costly. Nevertheless, baboons, cynomolgus monkeys, and rhesus macaques do have the unique cross-reactivity with human immunoreagents. NHPs are frequently used in xenotransplantation research, using pigs as organ donors. Pigs are usually the donors, as they can be relatively easy genetically manipulated, e.g., with knockout of the Gal antigen. As a result of the size ratio, baboons are frequently used as recipients in solid organ pig xenotransplantation. Smaller NHPs, such as cynomolgus macaques, play an important role in xeno-islet transplantation [38].

In contrast, pig and dog models are a satisfactory solution offering a relative ease of experimental handling, lower costs of breeding and handling, and in terms of pigs, they are socially more accepted [39]. However, the choices of available species-specific immunoreagents are still limited, but the increased demand is leading to the development of new reagents [39].

6. Kidney Transplantation

Large animal models are crucial in renal transplantation research, as there are only a limited number of in vitro models available due to the complexity of this organ [15][44][45].

As a result of the similar size of the kidney in swine and humans, the pig model is frequently chosen for research in the field of kidney transplantation and also for surgical training [15][46].

In addition, pigs do have about 80% of immune parameters in common with humans, and they have about 50–70% of neutrophils in their peripheral blood; this is comparable to human rates. As in humans, pigs also express CXCL8/IL-8, their macrophages do not express iNOS but IFN-γ, and LPS do stimulate IDO (indoleamine 2,3 dioxygenase) in macrophages. The expression of CXCL-8/IL-8 correlates with disease severity in pigs and humans in acute kidney injury [47]. The main advantage of the pig model is the human-like renal anatomic structure with multipapillary and multilobular kidneys [15].

Swine and nonhuman primate models play an important role in pharmacological preclinical studies regarding safety and efficacy [15].

In contrast to the suitability of pigs as a preclinical model stand some impairments such as the fast growth rates, which puts high demands on the housing and some anatomic specialties.

Regarding the operation, pigs have some unique anatomic structures and characteristics that a surgeon has to keep in mind. Especially, the narrow ureter and its mucosa is prone to edema, making this part of the transplantation challenging. Complications during surgery could also result from malignant hyperthermia, cardiac irregularities, peptic ulceration, and post-operative small-bowel obstruction [48][49].

7. Liver Transplantation

Large animal models for liver transplantation are extremely difficult models, as the prepared situation of acute liver failure results in a high mortality rate. In large animal models, the pig is the dominant species in use [50][51][52]. Still, these animal models often do not lead to translation into clinically relevant settings. The nature of liver disease plays an important role in this part, as the damage usually accumulates over years with severe impairment of both liver structure and function. The injuries developed in animal models are usually milder and occur in a timeframe of days or weeks. When it comes to the recovery of liver injuries, a realistic preclinical animal model is still lacking [53].

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